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Amelioration of aluminum toxicity on OM4900 rice seedlings by sodium silicate

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Silicon has versatile functions in plant biology, especially in plant defense and tolerance to both biotic and abiotic stressors. In this study, it was investigated if silicon could reduce the toxic effect of aluminum on rice. Aluminum was applied to OM4900 rice seedlings, and then their growth and sugar metabolism were determined. At doses of aluminum chloride of 100 and 200 µM, plant height was significantly enhanced and root elongation was reduced. The accumulation of photosynthetic pigments was tightly correlated with the aluminum concentration showing highest amounts at 400 µM. Sugar levels in roots and endosperm decreased upon aluminum addition. Aluminum toxicity in rice seedlings was relieved by addition of silicon in the form of sodium silicate. In the presence of 200 µM aluminum chloride, the addition of 100 mg/L sodium silicate to the hydroponic solution showed no contribution to plant growth or buildup of photosynthetic pigments. At this concentration, root development was not affected while it was at higher concentrations. Sodium silicate addition did not alter the reduction in sugar levels by aluminum. The presence of 200 µM aluminum chloride with or without sodium silicate in the growing solution significantly decreased silicon content in rice husks.

Key words: Cultivar, metal, root, silicon, sugar.

INTRODUCTION

Silicon is the second abundant element in the earth crust after oxygen (Epstein and Bloom, 2005). In soil solution, silicon is mainly present in the form of H_4SiO_4 and the concentration ranges from 0.1 to 0.6 mM (Epstein, 1994). In plants, silicon accounts for about 0.1 to 10% with a big variation between plant species (Epstein, 1999). The *Lsi* gene has been identified as the responsible gene for silicon uptake in rice (Ma et al., 2006; Yamaji et al., 2008). Rice plant absorbs silicon actively through the root hairs in the form of $Si(OH)_4$ (Ma et al., 2001; Ma and Yamaji, 2006; Ma and Yamaji, 2008). In rice, silicon is accumulated mainly in husk and stem (Currie and Perry, 2007). Silicon has recently been recognized as an important element playing multiple functions in plants (Epstein, 2009). It enhances silicon accumulation in rice stem and husk (Nhan et al., 2012), contributes to improve crop yield (Snyder et al., 2007), and particularly improves the tolerance of plants to biotic and abiotic stress (Epstein and Bloom, 2005). Silicon supplement ameliorates aluminum toxicity on maize (Barceló et al., 1993; Kidd et al., 2001; Wang et al., 2004) and wheat (Cocker et al., 1998) or tolerates to its poison on rice (Singh et al., 2011); the positive effect of silicon application on salinity stress has been studied intensively less is known on the beneficial effects of silicon supplement on relieving aluminum toxicity.

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Table 1. Effects of aluminum on growth and metabolites of rice seedlings of OM4900 cultivar.

$AlCl_3$(µM)	pH (cm)				RL (cm)	Photosyntheticpigments (µg/gFW)			TSS (mg/gDW)		Silicon (%)
	(2)	(4)	(6)	(8)	(8)	Chl.a	Chl.b	TC	Root	Endosperm	
0	31.1^{c}	60.2^{e}	70.5^{d}	83.7^{c}	156.3^{a}	323.5^{c}	114.6^{c}	1487^{c}	2.58^{a}	17.01^{a}	12.76^{a}
100	36.0^{b}	72.0^{d}	85.6^{bc}	117.9^{a}	136.8^{b}	300.4^{c}	116.9^{c}	1525^{c}	2.10^{bc}	13.15^{b}	10.46^{b}
200	36.7^{b}	75.9^{b}c	89.2^{b}	118.1^{a}	137.4^{b}	443.8^{b}	156.8^{bc}	2050^{bc}	2.04^{bc}	13.10^{b}	8.70^{c}
300	37.8^{ab}	73.0^{cd}	84.5^{c}	95.2^{b}	71.6^{c}	482.5^{b}	194.4^{b}	2556^{b}	2.00^{cd}	13.04^{b}	10.14^{b}
400	40.0^{a}	79.5^{a}	93.9^{a}	113.4^{a}	71.8^{c}	566.8^{a}	380.7^{a}	5058^{a}	2.13^{b}	12.05^{c}	11.00^{b}
500	38.0^{ab}	77.0^{ab}	84.9^{bc}	111.3^{a}	67.3^{d}	562.8^{a}	349.8^{a}	4641^{a}	1.89^{d}	10.40^{d}	10.28^{b}
P	**	**	**	**	**	**	**	**	**	**	**
CV (%)	4.41	2.94	3.37	4.36	2.58	5.98	10.36	9.63	3.24	8.22	7.52

**Significant difference at 1% level; DAS, days after sowingPH, plant height; RL, root length; FW, fresh weight; DW, dry weight; Chl., chlorophyll; TC, total carotenoids; TSS, total soluble sugars; CV, coefficient of variance. The numbers in brackets indicate the time points of the measurements at the DAS. Biochemical analysis was done with 3 replicates at each aluminum concentration. The PH and RL are the means of 27 plants. In a column; the numbers followed with the same letters are not significant different by Duncan's multiple range test.

It is well-known that aluminum toxicity is the most important growth-limiting factor for many crop plants in acid soil (Foy et al., 1978). Unfortunately, the major soil type in Mekong Delta in the south of Vietnam is acid sulphate soil and rice is the major crop in this region. Aluminum poison on rice in acid soil can be normally ameliorated by surface lime application but this is not an economical and physical way in reality (Hede et al., 2001). Therefore, the ameliorating benefits of silicon supplement in the form of sodium silicate to rice culture solution containing aluminum has been carried out to examine how much silicon could amend aluminum toxicity on rice seedlings.

MATERIALS AND METHODS

Popular rice cultivar named OM4900 which originated from Cuulong Delta Rice Research Institute was chosen for the experiment. Rice seeds were wetted and incubated for germination at room temperature (RT). The rice seedlings with the shoots of about 0.5 cm height were selected for hydroponics planting with the addition of aluminum ranging from 100, 200, 300, 400, and 500 µM of $AlCl_3$. Distilled water was used for the control treatment.

To test the efficiency of silicon in ameliorating aluminum toxicity of 200 µM on seedlings of OM4900 rice cultivar, sodium silicate was supplemented to the hydroponic solution with the concentrations of 0, 100, 200, 300, and 400 mg/L. The experiment was conducted with three replicates consisting of 27 plants each.

Chlorophyll a, b and total carotenoids in rice seedlings were analyzed at 45 days after sowing (DAS) by the method from Wellburn (1994). Photosynthetic pigments from 0.2 g of fresh material were extracted by mixing well with 10 mL of 80% acetone. The sample was left at RT under low light condition for 30 min and then 0.5 mL of this solution was diluted with 4.5 mL of 80% acetone. The absorbance at 663, 646 and 470 nm of the diluted solution was measured by a spectrophotometer (HEλIOSα, Thermo Spectronic, England).

To determine total soluble sugar levels of roots, the remaining endosperm of seeds were extracted three times with 5 mL of methanol 80% at 80°C for 1 h in a water bath. The extracted sugars were reacted with phenol – sulfuric acid mixture and quantified by measuring the absorbance of yellow complex at 490 nm (Dubois et al., 1956).

Silicon in rice husk was determined by the methods of Ma et al. (2002) and Mitani and Ma (2005). Dried sample (0.2 g) was mixed with 3 mL of 60% HNO_3, 3 mL of 30% H_2O_2, and 2 mL of 46% HF. The mixture was digested in an ultrasonic cleaner (Sonorex Super 10P, BANDELIN Electronic, Germany) for 10 min. Afterwards, the digested sample was diluted to 100 mL with 4% boric acid. The diluted solution (50 µL) was used for silicon determination. Distilled water of 1300 µL was added to the sample followed by the addition of 750 µL of 0.26 N HCl, 100 µL of 10% $(NH_4)_6Mo_7O_{24}$, 200 µL of 20% tartaric acid, and 100 µL reducing agent which was prepared by dissolving 1 g of Na_2SO_3, 0.5 g of 1-amino-2-naphthol-4-sulfonic acid, and 30 g of $NaHSO_3$ in 200 mL of distilled water. Formation of blue complex was determined by measuring the absorbance at 600 nm. For each treatment, the measurement was carried out with three replicates. For statistical analysis, SPSS 16.0 was used.

RESULTS AND DISCUSSION

The height of rice seedlings cultivar OM4900 was increased by aluminum up to a concentration of 500 µM $AlCl_3$ (Table 1). A concentration of 400 µM showed the strongest enhancement. After 8 days of exposure, the shoot height was 35% more on 400 µM aluminum in comparison to the control. In contrast, aluminum supply inhibited rice root elongation significantly especially at doses of 300 µM or more.

After 8 days of being exposed at 300 µM $AlCl_3$, rice root length was reduced to less than 50% of control roots. Data in Table 1 also reveal that root development of OM4900 rice cultivar is relatively sensitive to aluminum because at 100 µM, the reduction in root length was already clearly observed. This is an actual symptom because aluminum toxicity causes rapid inhibition of root growth (Hede et al., 2001), particularly in indica rice varieties (Watanabe and Okada, 2005). Inhibition of root elongation at 100 and 200 µM of $AlCl_3$ was similar and the later concentration of aluminum was chosen for further experiments.

In addition to shoot development, higher aluminum concentrations increased remarkably the amount of photosynthetic pigments in rice seedlings of OM4900 cultivar (Table 1). The highest enhancement was reached at 400 µM aluminum for both chlorophylls and total carotenoids. Interestingly, aluminum had stronger effects on chlorophyll b and carotenoids than on chlorophyll a. In addition, from the obtained data, it can be predicted that the biosynthesis of photosynthetic pigments in OM4900 rice cultivar at the seedling stage might not be enhanced when the aluminum exceeds the concentration of 500 µM.

Aluminum treatment might result in lower levels of soluble sugars in roots which might be responsible for the poor root development. The data in Table 1 show that indeed aluminum toxicity lowered the levels of sugars in rice roots and in endosperm. While growing in aluminum free condition, rice seedlings of this cultivar have always higher amounts of sugars both in root and endosperm. These data are in accordance with Le Van et al. (1994) results which stated that aluminum toxicity can rapidly inhibit root elongation and change carbohydrate components of squash seedlings' root. This was explained by inhibitory effects of aluminum on the conversion of starch in endosperm to sugars because the levels of total soluble sugars in root and endosperm are subjected to amylase activity (Gautam et al., 2010). As a result, the lower free sugars levels in endosperm might lead to a poorer translocation of sugars to the roots. The given data confirmed that aluminum causes a negative effect on the conversion of starch to sugars in rice endosperm. In addition, rice seedlings would have spent more energy to adapt to an unfavorable environment which might result in lower sugar levels.

As mentioned above, rice husk is a potential source for silicon. Many studies have shown that silicon is beneficial for many plants in fighting against heavy metal stress (Epstein and Bloom, 2005). We aimed to investigate if rice seedlings can exploit their constitutive endogenous silicon content to cope with aluminum at early stage. Our results support this hypothesis because the presence of aluminum in the cultivation solution led to reduced silicon content in rice husk at any concentration. It might also be deduced that aluminum speeded up the rice seedlings to use their own potential silicon availability in husk to overcome the difficult situation. Interestingly, the lowest remaining silicon in rice husk was observed at 200 µM aluminum.

After recognizing the toxic dose of aluminum on OM4900 rice cultivar, silicon was supplemented to the growing solution to examine its rescuing effect. In contrast to the effect of aluminum alone, treatment of rice seedlings with aluminum and silicon together did not lead to an enhancement of shoot height at all concentrations tested. In addition, the inhibition of root growth by 200 µM aluminum was prevented in the presence of 100 µM silicon. A recent finding recognized that silicate addition to the solution for rice seedlings contributed to an increase in their biomass (Gu et al., 2012). However, higher doses of sodium silicate display a strong inhibition on root development of rice seedlings. Our observations are in agreement with those of Gu et al. (1999) that aluminum inhibited root elongation and silicon supplement to the growing solution rescued Japanese rice cultivar (Koshihikari) from aluminum injury.

The presence of sodium silicate has also strongly affected the amount of photosynthetic pigments. In contrast with being exposed to aluminum alone, the combination between aluminum chloride and sodium silicate supplement deteriorates the positive effect of aluminum alone on enhancing photosynthetic pigments. Levels of photosynthetic pigments of seedlings treated with aluminum and silicon together were at least 2.5 times lower than that of control seedlings.

Sodium silicate supply to the growing solution in the presence of aluminum showed no effect on silicon levels in rice husk (Table 2). In this trial, the lowest silicon content in rice husk was also found at the 200 µM aluminum. An increase of silicon supply to the rice growing environment inhibited the utility of silicon or its release from the rice husk but there was no difference in silicon residual levels between the treatments.

Sugar levels decreased upon treatment with aluminum and silicon together similarly to treatment with aluminum alone. There was a little bit of variation in the analyzed data, especially in the levels of total sugars in the endosperm and roots. A possible reason could be that the low vigor of rice seed resulted over time of storage in negative consequences on sugars metabolism and amylase activity (Thobunluepop et al., 2009). Also, it will be interesting to analyze the activity of rice amylases under aluminum toxicity alone or in the combination with the addition of silicon because heavy metals can down-regulate hydrolysis ability of amylases during germination (Ahsan et al., 2007; Gautam et al., 2010).

In conclusion, silicon supplement with 100 mg/L of sodium silicate can restore root elongation of OM4900

Table 2. Ameliorating effects of sodium silicate on growth and metabolites of rice seedlings of OM4900 cultivar under aluminum toxicity.

$AlCl_3$ (µM)	Na_2SiO_3 (mg/L)	pH (cm)				RL (cm)	Photosynthetic pigments (µg/gFW)			TSS (mg/gDW)		Silicon (%)
		(2)	(4)	(6)	(8)	(8)	Chl.a	Chl.b	TC	Root	Endosperm	
0	0	32.7^{a}	61.5^{a}	81.9^{a}	106.3^{a}	130.1^{a}	398.6^{a}	148.7^{a}	1937^{a}	2.24^{a}	15.05^{b}	12.35^{a}
200	0	31.5^{ab}	54.7^{b}	74.3^{b}	97.6^{b}	110.0^{b}	375.3^{a}	132.0^{b}	1719^{b}	1.75^{ab}	12.28^{b}	8.18^{b}
200	100	31.2^{ab}	57.3^{b}	71.8^{b}	91.7^{b}	135.3^{a}	274.2^{b}	85.5^{c}	1115^{c}	1.29^{bc}	13.98^{b}	11.63^{a}
200	200	27.9^{bc}	45.9^{c}	61.5^{c}	65.9^{c}	79.9^{c}	200.0^{c}	61.5^{d}	807.3^{d}	1.95^{ab}	10.69^{c}	11.65^{a}
200	300	25.8^{c}	46.0^{c}	58.6^{cd}	63.9^{c}	67.0^{d}	181.8^{c}	54.9^{de}	718.0^{de}	1.54^{bc}	9.99^{c}	11.71^{a}
200	400	24.0^{c}	39.0^{d}	55.2^{d}	58.9^{c}	49.2^{e}	154.5^{c}	46.4^{e}	606.3^{e}	0.92^{c}	10.43^{c}	12.57^{a}
P		**	**	**	**	**	**	**	**	*	**	**
CV (%)		10.04	4.66	3.87	6.57	7.12	9.40	8.68	8.65	21.51	4.48	15.68

** and *Significant difference at 1 and 5% level; respectively. DAS, days after sowing; PH, plant height; RL, root length; FW, fresh weight; DW, dry weight; Chl., chlorophyll; TC, total carotenoids; TSS, total soluble sugars; CV, coefficient of variance. The numbers in brackets indicate the time points of the measurements at the DAS. Biochemical analysis was done with 3 replicates at each treatment. The PH and RL are the means of 27 seedlings. In a column; the numbers followed with the same letters are not significant difference by Duncan's multiple range test.

rice seedlings effectively in culture solution up to 200 µM aluminum. In future experiments, it will be tested if similar effects can be found for different forms of silicon. Finally, how much silicon from the culture solution has been absorbed by rice seedlings when they are exposed to aluminum toxic condition is another emerging question to be investigated.

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